apparent but may be due to small amounts of hydroquinone that resist the washings with acetone prior to the iodine number determinations.

With this stabilization of the iodine number there occurred a stabilization of the thromboplastic activity of the preparations. After the 25 day period the percent decrease in coagulation time produced by the control was only 44 while that of the hydroquinone-protected phospholipin was 80, 79, 79, 80 for the respective 0.5, 1, 2.5 and 5% hydroquinone-phospholipin preparations.

Summary. Hydroquinone in concentrations as low as 0.5% protected a thromboplastically active phospholipid preparation from being autoxidized for a period of at least 25 days. With this protection against autoxidation there was a protection against loss of thromboplastic activity.

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Metabolism of Isocysteine, Labeled with Radiosulfur (S^{35}), in the Rat.

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The administration of isocysteine hydrochloride, α-thiol-β-amino-propionic acid, to rabbits results in a large excretion of "extra" sulfur in the urine.[1] Almost all of this extra sulfur is in organic combination; a large fraction of it is in the form of disulfide which is presumably isocystine, since it does not give the Sullivan[2] reaction. When isocysteine is fed to rats maintained on a diet low in proteins (6% casein), the animals' growth is not increased. The present investigation was undertaken to determine whether the sulfur of isocysteine is oxidized to sulfate by the rat.

Procedure. Isocysteine hydrochloride was prepared from α-bromo-β-alanine and labeled sodium disulfide according to the method of Schöberl and Braun.[3] Five grams of α-bromo-β-alanine hydrobromide and a solution containing 325 mg of disulfide sulfur containing S^{35} equivalent to 130,000 ± 2500 c.p.m. were used in the synthesis. The sulfur in an aliquot of the solution (in 0.1 N sodium hydroxide) of sodium sulfide which was used to prepare the disulfide for the synthesis was oxidized by alkaline fusion. A small amount of carrier was added to the resultant sulfate, and the mixture was precipitated as barium sulfate. This was washed by repeated centrifuging, first with 0.001 N hydrochloric acid and then with water, and transferred as a slurry in 70% alcohol to a tared counting cup, dried, weighed, and counted[4] using a 5/mg/cm² end-window G-M tube and a scaler. Counting was continued for a sufficient time to insure an error of less than 2%. The sulfate later obtained from the specimens of biological material was precipitated, washed and counted in the same way as the sulfate from the original sulfide-sulfur solution except that no carrier was added. All counts were corrected for self-absorption and for decay.

The isocysteine hydrochloride as originally isolated was grossly impure and was purified by conversion to and recovery from its mercaptide. The final yield was 0.88 g (25%) and contained 24% of the initial radioactivity.

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1 Wingo, W. J., and Lewis, H. B., *J. Biol. Chem.*, **1946, 165**, 339.

2 Sullivan, M. X., and Hess, W. C., *Pub. Health Rep.*, U.S.P.H.S. supplement No. 86, 1930.

3 Schöberl, A., and Braun, H., *Ann. Chem.*, 1939, **542**, 274.

4 Dziewiatkowski, D. D., *J. Biol. Chem.*, 1945, **161**, 723.

Its sulfur content was 19.4% (theory is 20.34%).

In each of 5 experiments, 100 mg of isocysteine hydrochloride (3627 c.p.m.) were given to young adult rats (140-168 g) from the McCollum colony at the Johns Hopkins University. In 3 cases the isocysteine hydrochloride was given by stomach tube; in 2 cases it was given by intraperitoneal injection. The rats were fasted for 24 hours before being given the compound; afterwards they were kept, still fasting, for 24 hours in individual metabolism cages. They were then killed with chloroform; and the radioactivities of the sulfur fractions of the urine, the feces, and the intestinal contents were determined. If the bladder contained urine at autopsy, this urine was added to the 24-hour specimen from the metabolism cage. Samples of the inorganic sulfate-sulfur, the total sulfate sulfur,[5] and the total sulfur[6] of the urine were isolated as barium sulfate. The feces and the intestinal contents were hydrolyzed with sodium hydroxide for 6 hours on a steam bath. An aliquot of each of these samples was acidified to Congo red paper with hydrochloric acid, filtered, heated for two hours longer, and extracted 3 times with ethyl ether; the total sulfate in the aqueous phase was then precipitated with barium chloride. Other aliquots of the alkaline hydrolysates were oxidized with sodium peroxide according to Bailey's method,[7] and the sulfate thus obtained from the total sulfur was precipitated as barium sulfate. The barium sulfate from these samples was isolated, weighed and counted according to the procedure already outlined.

Results. The data in Table I indicate that isocysteine hydrochloride is fairly well absorbed from the intestine of the rat within 24 hours, since in 2 of the 3 experiments in which the compound was given by stomach tube, 3% or less of the radioactivity of the dose given was recovered in the feces, and in 2 of the 3 experiments 5% or less was recovered from the intestinal contents. It is

TABLE I.
Excretion of Sulfur and Radioactivity in 24 Hours by Rats Following Administration of Isocysteine Hydrochloride Containing S^{35}.

Rat	Urinary Sulfur						Fecal sulfur				Intestinal sulfur			
	Inorg. SO_4-S		Total SO_4-S		Total S		Total SO_4-S		Total S		Total SO_4-S		Total S	
	mg	%‡	mg	%‡	mg	%‡	mg	%‡	mg	%‡	mg	%‡	mg	%‡
2*	5.2	9.5	5.5	9.9	21.4	66.9	0.5	0.16	20.0	2.7	1.6	0.45	4.8	2.7
3*	4.9	5.8	6.0	8.0	20.9	57.5	0.5	0.45	5.3	3.0	2.4	0.79	—	—
4*	5.2	6.6	6.0	8.7	21.4	54.2	1.3	1.1	11.8	14.6	1.1	0.53	8.8	5.0
5†	5.5	4.6	6.0	6.3	21.4	52.6	0.5	0.17	4.1	0.21	0.5	0.00	11.9	1.1
6†	6.3	5.2	6.6	7.5	24.4	52.4	0.5	0.09	4.4	0.80	0.4	0.22	8.5	1.3

* Given 100 mg isocysteine hydrochloride (3627 counts/minute) by stomach tube.
† Given 100 mg isocysteine hydrochloride (3627 counts/minute) intraperitoneally.
‡ % is noted as % of total activity administered.

[5] Folin, O., *J. Biol. Chem.*, 1905-06, **1**, 131.
[6] Denis, W., *J. Biol. Chem.*, 1910-11, **8**, 401.
[7] Bailey, K., *Biochem. J.*, 1937, **31**, 1396.

of interest that a small but definite fraction of the radioactivity was recovered as sulfate and as organic sulfur from feces and intestinal contents of rats given the compound intraperitoneally. The activity of the feces in these experiments could have been due to contamination with urine despite precautions employed to prevent such contamination; this could not be true in the case of the intestinal contents, since these had never been in contact with urine.

It is apparent that, while a small portion of the sulfur of isocysteine hydrochloride given to rats either by stomach tube or by intraperitoneal injection may be oxidized to sulfate and excreted as such, a much larger fraction is still bound in organic combination when it is excreted. The rat, therefore, reacts similarly to the rabbit in that it does not readily oxidize the sulfur of isocysteine to sulfate.

Quantitative differences between the two species may, however, exist. The rats employed in this investigation excreted in the urine, within 24 hours, 54 to 67% of the S^{35} administered as isocysteine by stomach tube. The rabbits employed in the earlier study[1] excreted, within 24 hours, "extra" sulfur equivalent to only 29 to 37% of the dose given by stomach tube. On the other hand, the rabbits excreted "extra" sulfur equivalent to 76 to 98% of the dose when isocysteine was administered subcutaneously; rats excreted only about 52% of the S^{35} of isocysteine which had been injected intraperitoneally. The metabolic fate of the S^{35} not found in the excreta or intestinal contents of these rats cannot be ascertained from the present data, but presumably it was still contained in the tissues.

Summary. 1. The oxidation of the sulfur of isocysteine by the rat was studied with the aid of isocysteine containing S^{35}.

2. It was found that only a small amount of S^{35} was excreted as sulfate in the urine within 24 hours after administration of the compound by stomach tube or by intraperitoneal injection. A much larger portion was excreted as organic sulfur. A very small fraction of the S^{35} was recovered in the feces and intestinal contents of the rats.

3. The results of the present study are compared with those of an earlier investigation in which rabbits served as subjects.

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Streptomycin in the Treatment of Leptospira Carriers. Experiments with Hamsters and Dogs.*

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The carrier problem dominates the control of canine leptospirosis. Dogs remain urinary shedders for from 2 to 6 months after recovery. With the introduction of chemotherapy, the risk of creating carriers and thus spreading leptospirae is greatly increased.

Penicillin and streptomycin have been found to inhibit growth of leptospirae *in vitro* and to be effective against experimental leptospirosis in guinea pigs, mice and hamsters. Shih Lu Chang[1] observed that *Leptospira icterohaemorrhagiae* disappeared from the blood of guinea pigs 3 to 5 days after 2 daily injections of aqueous solution of penicillin in total daily doses of 800 units. A

* Aided by a grant from the National Canine Research Foundation.

1 Chang, S. L., *J. Clin. Invest.*, 1946, **25**, 752.